Borgnia, Mario 2020

Dr. Mario Borgnia Oral History

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Dr. Mario Borgnia

Behind the Mask

December 10, 2020

Gabrielle Barr: Good morning, today is December 10, 2020, and I have the pleasure of speaking with Dr. Mario Borgnia. Dr. Borgnia is the Director of the Molecular Microscopy Consortium at the National Institute of Environmental Health Sciences (NIEHS). He's going to talk about his COVID-19 research. Thank you very much for being with us.

MBorgnia: You're welcome. Thanks for having me.

GBarr: So, to start off, why is the S protein so important to cracking issues around developing therapeutics for COVID-19?

MBorgnia: The S protein is a sort of a swiss army knife for the virus. In general, many viruses have proteins similar to this S protein. The virus, in order to propagate, needs to be inside a cell; it gets into a cell and produces proteins that assemble into a shell surrounding its genetic material; and then it has to go outside of the cell to find another one. That's the way it propagates. Viral particles packing genetic information go from cell to cell using them cells as factories to produce more viruses. The problem for the virus is that whenever they are outside of the cell, its proteins are recognized as foreign making it susceptible to attack by the immune system.

Viruses like SARS-CoV-2, the agent of COVID-19, solve the problem by carrying a piece of the “skin” of the cell, the cell membrane, as they bud out. Essentially, these viral particles get out of the cell surrounded with a piece of membrane that works as cloak rendering the viral proteins “invisible” to the immune system. The problem is that in order for the virus to enter a new cell it now needs to go through two membranes. But first, it has to recognize the target cell. This is where the S protein comes into play. During viral assembly, S protein gets inserted in the portion of the cell membrane that the virus carries as a cloak. S has the dual function of recognizing the next target cell and fusing the cloak with its membrane.

Now the problem is S is a protein of viral origin and because it is distinct from the human proteins in the membrane of the cell, it now becomes a target for the immune system. So, it's a problem that actually finds a solution and then finds a problem and so on. The virus is all the time fighting the immune system by mutating that envelope glycoprotein or by disguising that envelope or protein in some way. But that's the only hope for the immune system: to find the virus. So that's why this protein is so important. If we can teach the immune system to recognize this protein, then we can actually create a vaccine to block the activity of that protein which is the fusion machine that allows the virus to get in the cells. Then we can prevent the virus from infecting.

GBarr: So, is that why it's so critical to look at both the protein and the environment? I know that's what your research is doing.

MBorgnia: The protein in its environment… Okay, let's backtrack a bit. S protein has a stalk that anchors it to the membrane. Proteins of this type are very difficult to study because the membrane is not so easy to isolate as an intact environment. One way scientists have solved the problem is by engineering a truncated stalk that preserves the shape but lacks the membrane part. Now in addition to being an anchor and containing part of the fusion machinery, this membrane portion may have some implications in how the external part looks. One of the questions is: Is there any regulation? Is there any exchange of information between the inside of the viral particle and the outside through the transmembrane section? In order to answer these and other questions we need a system that preserves the membrane portion. Is it absolutely necessary? We don't know, but we cannot dismiss the possibility that the membrane plays a role.

GBarr: Oh, that's interesting. Can you describe your research in lay terms? I know you're looking at the structure and conformational landscape of the native S protein on the SarsCov-2 protein and its complexes with neutralizing antibodies and drugs.

MBorgnia: I started working on a similar protein about 12 years ago and that's the envelope glycoprotein of HIV called Env. We do not always think of [HIV] as a pandemic, but actually that was the pandemic of my 20s. I mean when I was in my 20s, that was “The Pandemic” and it continues to be a pandemic. We are now interested in the communication between the internal and the external parts of the virus. One of the things we know is that the HIV virus buds out of the in an immature form and matures outside of the cell. The immature form does not seem able to fuse with a target cell likely involving the external portion of Env. The maturation process involves changes inside the membrane. How are these changes communicated to the external part? We established a methodology that allows us to determine the shape of Env in the context of the membrane. This allows us to study maturation intermediates and to look at changes that occur when you mutate the protein. I’m also curious about the mechanism of fusion.

We have quickly repurposed this methodology to study S protein. One important application is to look for molecules that could neutralize the spike in different ways. This is done around the globe as many researchers now are working on these. They're looking for antibodies that can recognize S protein. One particularly interesting class are antibodies from llamas, the llamas in South America, who produce tiny antibodies called nanobodies. So what we do, is we use our method to look at the shape of these complexes between S protein and different nanobodies. Our work for the project that you probably read about is essentially setting up a pipeline so that different collaborators from NIH can come and say, “Okay, I want to see how this nanobody interacts with S protein, or I want to see how this drug interacts.” We establish an assay that is not widely available to actually answer those questions. That is within the context of an interest in developing that type of assay and, on the other side, an interest in understanding how this protein works, how this system works.

GBarr: Are you testing the neutralizing antibodies in drugs, right now, and with your group or are you just producing the assay for other groups to test?

MBorgnia: Our collaboration with Anton Simeonov and Matt Hall at the National Center for Advanced Transfer Sciences, NCATS, is a good example. Ying Fu and Alex Renn at NCATS have produced this library of nanobodies using an amazing method called phage display libraries. They use viruses that infect bacteria, called phages, in order to produce proteins that will recognize things. These engineered viruses display on their outer side a portion of a protein of interest that can stick to something, in this case they carry a nanobody. Now what they do because they have this in a simple virus, they can do a whole lot of mutations on that sticky part and those mutations are like lottery numbers in a way because you combine many different mutations to get many different shapes that stick to different things. In a single test tube, you can have billions or trillions of viruses that have that variable region with a unique shape. Now you can take the thing that you want to test, in this case the spike protein, the S protein, then you can stick the viruses to it. Put the virus on top of a surface where you have S protein immobilized and then wash. Whatever sticks has a unique nanobody that is encoded in the DNA of the virus displaying it, so you do that several times and then eventually you end up with a bunch of viruses that encode for something that sticks to the spike, the winning lottery numbers.

After that process, what our collaborators come up with are distinct nanobodies. In this case, that's the sticky part. They give them to us and then we look at how they bind to the spike, where they bind, where they bind in the place where the spike acts on the cell to recognize itself, the ACE2 receptor. It is probably quite known by the audience as the receptor for the SARS-CoV-2 virus. Then we look at the shape.

So, this is a highly collaborative work where colleagues at NCATS use this phage display library to identify and produce the nanobodies. Our colleagues Robert Petrovich and Tom Stanley at the NIEHS Protein Expression Facility and Negin Martin at the Viral Vector Core produced quantities of S-protein. Then we look at the structure and we say, “Okay, this one, maybe with some modification will be more effective.” They go on to produce the modification and we iterate over that so the hope, in this particular case, that at the end of this process we have a protein we can produce that can be used in an inhaler, for instance, to block the virus inside the lung of a person, or we can produce a molecule that has been now engineered to block the virus that can be injected.

This is not the first use of nanobodies and phage display libraries. But our colleagues at NCATS use scaffold at the beginning of the process, a nanobody that was humanized in the sense that it's now compatible with humans and does not elicit an immune reaction, the original scaffold has been used before in an anti-cancer nanobody. So now, because they're modifying only the variable portion of the nanobody, it's likely to be easily acceptable by the FDA as a treatment. Connecting the pieces—when the President [Donald Trump] received eight grams of antibodies, they were coming from larger antibodies or human antibodies produced by a different system. We are producing something similar, and we can make it in bacteria which is also very good because you can produce it essentially like yogurt, whereas the other system has to be produced in a much more complicated environment. But the answer to your question is we are a part of a larger research program and we are collaborating with many groups and offering this portion of the analysis.

GBarr: So, in addition to NCATS what other groups are you working with?

MBorgnia: We are working with a group at NIDA [National Institute on Drug Abuse] that is looking into other relations of this spike protein that may have something to do with the fact that susceptibility is different between males and females, for instance. Much of this research started because we have been collaborating with Priyamvada Acharya and Bart Haynes at the Duke Human Vaccine Institute. Priyamvada was working on the soluble portion of the protein, the one that—by the way, this S protein is the message encoded by the mRNA, the messenger RNA that the Moderna and Pfizer vaccines are using. So actually, understanding how the product looks is important.

Priyamvada is interested in developing more effective vaccines and she uses what we call the ectodomain, the part that doesn't have the membrane portion. We can look at both as we can look at the ectodomain and we can look at the protein in the virus. So, we started the work with her—we were working on HIV before, that was a long-term collaboration. We are also working with Eric Freed at NCI. Eric is interested in the maturation of HIV but, when we realized we had something to offer with Eric, with Priyamvada and others, we rapidly switched from the HIV virus to this SARS-CoV-2, and we had a steep learning curve to understand the differences and how to how to look at those.

GBarr: What has it been like? They are very different viruses, I guess, like Sars-CoV-2 and HIV. What has that learning curve been like? What did you have to do to get up to speed and transition?

MBorgnia: Initially our simplistic view was okay. They are looking at a particular protein and we have a system that allows us to look at those viruses, so let's just produce them right now. They are different; they are not terribly different in the sense that the mechanism that we are looking at is fairly similar. We found some hurdles, so the places in the cell where the virus is produced are different. The places in the cell where this envelope glycoprotein needs to be inserted are different. So initially we were chasing after a place which didn't have any of the proteins. We had to actually figure out what we needed to do to actually have the part that did have the protein.

As I said, the protein has three portions: an external portion or ectodomain, a transmembrane portion, and then a cytoplasmic portion. The cytoplasmic portion determines where in the cell the protein is targeted. Now we are using, in collaboration with the viral vector core at NIEHS—the viral vector core's primary function is to produce special viruses that can be used to target special cells in systems to study disease. They know how to pack viruses and they know how to pack spike, so we went to them and said, "Hey, we want a virus that packs this spike, but it won't make me sick because it's not Sars-CoV-2. We can look at that virus." Negin said, "Okay, no problem, we'll use a lentivirus which is a non-infections virus in the family of HIV so it's not the coronavirus." She produced it, we put it in the microscope, and we don't see any spikes. The problem is that lentiviruses bud at the membrane and coronaviruses bud inside, in a different organelle, and then they traffic to the membrane. So, you have to actually look at the trafficking, the targeting, of that protein. If your protein goes to the membrane, then it will be incorporated into the lentivirus, and if it goes to the internal machinery that produces certain proteins, it won't be incorporated, because the lentivirus buds at the membrane. We needed to actually change the signals in the cytoplasmic portion of S-protein so that it will go to the membrane.

GBarr: What organelle does COVID-19 come from?

MBorgnia: It's actually in some of segment before the Golgi apparatus. I'm not exactly sure but there is a retention signal that will retain the protein in the endoplasmic reticulum. Then the assembly will occur in an intracellular organelle that will essentially go out. HIV assembles on the membrane. So, the target in HIV is a whole lot of envelope glycoprotein expressed in the membrane and that leads to other things; for instance, HIV infected cells fuse with other cells because they essentially have the machinery. They produce these syncytia that are not present in, or maybe they are present but they're not so common, in corona. Interestingly, this is a digression, and maybe you want to keep it or not, but over the eons humans have incorporated viral proteins in their genomes. So here and there, very, very rarely, a virus infects a cell that ends up being a germ cell. It ends up being a cell that can transmit the viral proteins from generation to generation. There is a particular protein called syncytin. That protein appears in some mammals, not in all mammals, and is involved in producing a syncytium, a fusion of cells, in the placenta of mammals. Without that protein the mammals that use it will not produce offspring. They will be sterile. Syncytin is a protein of viral origin of the same type as the protein of HIV and the protein of coronavirus but now its gene is part of the human genome. Without that human gene we could not produce more humans. We actually inherited the protein from a virus at one point in evolution that now is essential for us to reproduce.

GBarr: That's great. That's really interesting.

MBorgnia: I think that's amazing. Those things since we look at viruses as our enemy because to a great extent, they are. But on the other side they can be part of our ancestry, if you wish.

GBarr: Can you talk a little bit—I know that you do a lot with a certain method that I don't know how to pronounce exactly—can you talk a little bit about that, like what unique understanding it provides? Can you walk us through the process of you using it?

MBorgnia: Sure, so the cryo-electron microscopy—the word comes from "cryogenic," which is related to cold, electron microscopy—so we use an electron microscope that is kept very, very cold to produce images. Now, those images are in two dimensions. They are close to what we call a projection image, so if you take a three-dimensional object and you irradiate it and look at the image that comes out of that object, then you can get some information about that object. We do that all the time, for example, you go to your dentist and you get the picture of your teeth. Now you don't get a regular picture of your teeth, you don't see the surface of the teeth, you see through the teeth, so you will see a tooth that actually has a shape and what you see are gray values. It depends on how the film was developed, you will get the darker areas where the x-rays pass freely and the brighter areas where the x-rays were delayed, or the other way around, a positive or a negative. Those images carry information all across the tooth or across any object that you're imaging with x-rays for instance. Why do we use x-rays in those cases? Because x-rays go through most materials, they are transparent to x-rays. Now because it's a projection image, the image carries information of all that happened to those x-rays as the moved through the material. If you combine this information of the density of the object when you look at pictures from different sides [you can] actually get a three-dimensional description of the structure. The most common application that people may be familiar with is a CT scan. In a CT scan what you do is take pictures of a patient from different angles and then you combine them to get the 3D view of the patient that allows you to see internal organs. The issue is now that in order to get a CT scan of a protein, you have to magnify the image. Magnifying the image with x-rays is not so easy, but with electrons it's easier. We use an electron microscope to essentially do a CT scan of a virus or a molecular complex. It's a little bit more complicated than that, but we combine different views computationally to get the structure. This process requires that the technologies are at bleeding edge of several technologies. You have on the one side electron microscopy that involves high vacuum, high voltage, and having a high vacuum, high voltage system that is stable is not so simple. It combines cryogenics because biological materials do not really like to be irradiated by electrons, so you have to actually keep them really, really cold.

GBarr: How cold does it have to be, out of curiosity?

MBorgnia: It has to be as cold as liquid nitrogen at about 100 kelvin which is about 200 degrees centigrade below zero. There is another problem: in order to freeze things to that temperature—first of all, if you freeze living materials and you freeze them in the improper way, you get all this crystallization that actually breaks down the materials. There was a whole lot that was done over the past few decades to actually preserve the materials all the way. The technique actually was not very useful to get the structure until about five or six years ago when we changed the methods. We got much better detectors that can actually catch the signal much better. Then we began to be able to actually get structures and useful information that people now can use to get the information about how antibodies or nanobodies.

GBarr: That's very interesting. What metrics are you all using to assess your progress?

MBorgnia: Methods to assess our progress? The first question will be, “What is progress?” That depends on goals and on interests. In essence when we talk about SARS-CoV-2 progress is finding something that cures COVID-19 too.

We're not dealing directly with that. We are really very indirect and quite removed, so it's very difficult to say, “What is progress relative to that?” For us progress is being able to produce a three-dimensional map of whatever we are looking at and we assess what we call resolution. Resolution is the ability to see two things that are at a certain distance as two separate things. So in order for you to be able to describe something you need to be able to tell the features apart. As an example, if you are looking at an animal and you want to know whether that animal is a cow or a horse, you may be looking for horns. In order for you to be able to see the horns, you should be able to see two different things that are separated about four inches. If you can tell things that are four inches apart, then you have enough resolution to see the horns. If you cannot tell that or you cannot tell whether you see a horn and an ear or just an ear, you may mistake a cow for a horse. The resolution thing is that ability to find things at a particular distance.

Now when we look at the interesting aspects of interactions between molecules, where we are looking at the distances between atoms, we are now looking at atomic resolution. Our primary assessment of progress is, “Can we see the interaction, and can we see the interaction so we can describe it at the atomic level?” Why the atomic level? Because that's where pharmacological agents differ from each other, so a molecule that has a particular shape will go and stick to a particular pocket, a particular area that has a shape that is complementary. Much of the development of drugs, for instance, is based on modifying a particular chemical so that it, for example, kills a bacterium but does not kill a human because it's targeted similarly. Understanding that's the criterion for progress: If we can actually get to that resolution.

Now the problem is that imagine you are trying to get the structure or the shape of the legs of the cow and the horse, but they are running, so one of the problems is that then you get the blur of motion. So you get the shape of the body but you don't get the shape of the legs. Then we go into strategies to freeze the movement in the sense that we begin taking pictures, and now taking a picture, if you can image it properly, we can take a picture, and try to classify the picture of the body with the legs extended in one direction from the picture of the bodies of the legs in the other. So progress at that level…okay, it seems this guy is actually looking for progress at the very level that is completely unrelated with somebody actually being a hospital, unfortunately. Eventually it could be, so you know that's why we…

GBarr: Have you developed any new strategies for freezing things with this virus with your work?

MBorgnia: Not really. What we are doing… so methodologically one of the problems is that electrons give a very noisy picture and that's because if you use too many electrons to get a very clear picture, you destroy the biological material. The electrons are very bad for biological materials, so we need to use a very low dose and then we get a very—it's like when you take a picture with your iPhone in a pitch-black scene, you get very little signal. You get a very grainy signal. For instance, in the tomography portion where we combine images of the same virus, the problem is that the method is very slow. Since we were already working on an HIV-based project on developing faster methods, so one of the things that came up in the past few months that was already under development is that we were able to increase the speed of data collection by tenfold. Where over a weekend we were getting 100 tomograms that we could average to get some structure, now over a weekend we can get a 1000, which changes the picture. But those are incremental changes, so in the technology we are not changing a lot, we are learning ways of concentrating our attention in particular portions of the protein. We are learning. The amazing thing about science is that you're looking at the problem and things are happening that may actually help you look at different problems after a while.

GBarr: Have there been anything surprising that you have seen or learned so far?

MBorgnia: Not in what we are doing, not amazingly surprising and nothing that is flashy. I don't think I can, other than understanding that the targeting, as I said, the retention signals, so far we haven't come but the excitement is pretty high. The thing that we learn is how great the environment is that we can actually change directions and change course. It's more of a sociological managerial thing than scientifically. But the ability of NIH to actually turn around and begin looking at a problem that was on the back burner or actually looked at by very few people, I think that's the most surprising thing. So, it's not scientific, it's a different thing.

GBarr: On that note, what has been your role with this project, and can you talk a little bit about that as well as your team that you're working with?

MBorgnia: Our role technically has been applying the methodology that we had to a new problem, that's technologically or technically. I did many things in the beginning. I remember it was Friday the 13th we received the first sample of corona virus-related protein, and on Tuesday, the 17th of March, we actually got the first structure. Others had actually obtained the structure, but it gave us the confidence that we could go and now look at antibodies and at other things. But at that time the feeling was, Okay, we are going to lock down, we are going into a moment when people are going to be in their homes trying to figure out what to do.

One of the things that I began doing with others was trying to create an environment where people could actually participate in the research and have information on the scientific level, especially for scientists at NIH. I thought that would be a useful thing to do so I became part of the COVID-19 Scientific Interest Group at NIH that was involved in setting up very quickly the Wednesday Afternoon Lecture Series into the COVID-19 Lecture Series and began inviting people who had something to say. The feeling was that “Okay, now we have this machinery at NIH that is capable of doing research at the very high end and it's not concentrated on COVID-19.” In that context my role, my vision, my ambition was to push people to begin working on this, so that serendipitously at least something will come out of this—and the silver lining is what came out of this is a whole lot of collaborations. We began together with others organizing all sorts of things. The result was the Viral Vector Core produced viruses that we could use in the laboratory without exposing ourselves to COVID-19. That was used by Stavros Garantziotis who was looking at the air-cell interface in lungs and they could use this virus that we were producing. So, the primary role was to run this pipeline that allows you to get structures but, in that endeavor, we began connecting people. We became part of a network connecting people and I think that was probably more important in the long run than what we did with our microscope and our techniques. That idea of okay, let's open and let's include serendipity as one of the magic sauces that may actually get to some solutions.

GBarr: That's very interesting. Have you mostly been working at home, on campus or a combination?

MBorgnia: Personally, I actually have been working at home and I've been actually carrying out meetings like this one with different groups and for different purposes for the past nine months. Actually, I think I went to the lab four or five times. In the meantime, now I have a very talented group of people, and younger people, who are probably at a lesser risk, actually were the ones operating the instrumentation we have. I always had an interest in automation and in the ability to remotely access the resources and we were well set up for that.

The next day we were able to actually control the microscope from our homes. That was already in place because we ran kind of a 24/7 operation where somebody's always looking at the microscope and, having been there at the side of the person who has to be in charge of the microscope, I actually was always interested in developing the ability of doing it from home. So we are working a whole lot from home and we have people who are there, but they don't need to be a long time; they just go for a couple of hours to load the microscope and then we continue working from home. It's very interesting and very useful.

GBarr: What have been some personal challenges that you've had with COVID-19 as well as some opportunities that have come up for you?

MBorgnia: Personally, I mean, within my professional life or in general?

GBarr: It's your choice. It's what you choose to share.

MBorgnia: I think that this is something many have said: it has been an accelerator of many processes. This ability of talking to you over Teams or Zoom or Webex or whatever has created the possibility of running meetings much more efficiently. We used to scramble with computers connecting things to the projector to be able to see things; now we share our screens, better than meeting in person. In that sense you don't get the community of humans in the same place chatting to each other. In terms of efficiency that has been very useful. So overall I must say I'm fortunate that actually I had the right configuration. I know that there are way too many people that are not so fortunate and that's the sad part. I don't think it has hit me individually as much in a negative sense, but I can feel the negative effect in general. You know I just happen to have grown-up kids and so it hasn't affected us too much, but I know people are suffering.

GBarr: This is a fun question. How has COVID-19 impacted your winter holiday plans?

MBorgnia: My winter holidays. That's a good question. My winter holiday plans were to go to Florida and spend some time on the beach. I don't know yet how it's going to impact me because I have a mobile home. I can actually stay in my home and probably even continue working which is no holiday. But I think the smart and the safe thing to do is to stay put for another few months. It's going to be seeing family [remotely] which also has its advantages and disadvantages. We don't share the food. We can turn each other off when we are not too happy.

GBarr: Is there anything else you would want to share as an NIH scientist or as a person living through the pandemic?

MBorgnia: I think I must say that the support of NIH has been great for us. I think this is important to say because it's important to complain when things are not done right and it's important to praise the leadership when things are done right. I think that the leadership was exceptionally supportive at many levels starting from the Scientific Director all the way to the environment and colleagues who did the research with us. The understanding also that people needed space and help with flexibility, so I'm very grateful for all that. It's as I said again, I feel like we are probably the fortunate ones.

GBarr: Thank you very much for providing your insight. I hope that you and your family and your team continue to stay safe. Thank you.